

# Banana xanthomonas wilt and potato bacterial wilt in Rwanda

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Biology, risk factors and farmers' awareness

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# Banana xanthomonas wilt and potato bacterial wilt in Rwanda. Biology, risk factors and farmers' awareness

## Abstract

Banana (*Musa* spp.) and potato (*Solanum tuberosum*) are important food and cash crops worldwide as they contribute to food security and income generation for farmers. Despite their importance, banana and potato do not reach their potential production due to a number of limitations, including pests and diseases. In Rwanda, banana xanthomonas wilt caused by *Xanthomonas campestris* pv. *musacearum* and potato bacterial wilt caused by the *Ralstonia solanacearum* species complex (RSSC) are the major diseases of banana and potato respectively. These disease-causing bacteria have similar transmission means and the two crops are vegetatively propagated. Little is known about these diseases in Rwanda. The aim of this study was to investigate the biology, risk factors and farmers' awareness of banana xanthomonas wilt and potato bacterial wilt and how they affect disease occurrence in Rwanda. Surveys revealed that both diseases were present in major and minor growing areas for potato and banana. Banana xanthomonas wilt incidence varied between 27 to 77 % in 2015 and from 26 to 86% in 2016. Disease incidence ranged between 5 and 24 % in 2014 for potato bacterial wilt. These records are high considering the importance of the crops. The farmers' knowledge and implementation of management strategies were insufficient for both diseases, due to inaccessibility to adequate information. Cultural practices such as mono-cropping, intercropping, wide spacing and avoidance of sharing tools were highly associated with low potato bacterial wilt occurrence ( $p < 0.05$ ), whereas dense spacing, intercropping and beer bananas were linked to high occurrence of banana xanthomonas wilt ( $p < 0.05$ ). The population of the RSSC causing potato bacterial wilt in Rwanda is dominated by phylotype II strains (*Ralstonia solanacearum* emend. Safni). The isolates used in host range and cultivar susceptibility tests to the bacteria, *X. campestris* pv. *musacearum* and *R. solanacearum*, infected only banana or potato respectively and their close relatives, and all the inoculated cultivars were susceptible but at relatively different levels. These studies have improved our understanding of banana xanthomonas wilt and potato bacterial wilt in Rwanda, and provided important insights towards development and communication of sustainable management approach for the diseases, which in turn will improve food security in the country.

**Keywords:** bacterial diseases, detection, infection pathways, management, *Ralstonia solanacearum*, *Xanthomonas campestris* pv. *musacearum*.

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# Xanthomonas visnesjuka hos banan och mörk ringröta i Rwanda Biologi, riskfaktorer och odlarnas perspektiv

## Sammanfattning

Globalt sett är banan (*Musa* spp.) och potatis (*Solanum tuberosum*) viktiga grödor, både för direkt konsumtion och för försäljning eftersom de bidrar till livsmedelsäkerhet och inkomster. Trots deras stora betydelse når man sällan de förväntade produktionsmålen på grund av faktorer såsom växtsjukdomar och skadedjur. I Rwanda är xanthomonas-visnesjuka hos banan, orsakad av *Xanthomonas campestris* pv. *musacearum*, och mörk ringröta i potatis, orsakad av artkomplexet *Ralstonia solanacearum*, den viktigaste sjukdomen hos respektive gröda. Dessa sjukdomsalstrande bakterier har liknande spridningsvägar och båda grödorna förökas vegetativt men inte mycket är känt om dessa växtsjukdomar i Rwanda. Målet med denna studie var att undersöka biologin hos, riskfaktorer för och odlarnas medvetenhet om dessa sjukdomar och hur detta påverkar förekomsten i Rwanda. Fältinventeringar visade att båda sjukdomarna fanns i alla odlingsområden där potatis och banan produceras, även där de odlas i mindre omfattning. Förekomsten av xanthomonas-visnesjuka varierade mellan 26 - 86% på de undersökta gårdarna under 2015 - 2016. Mörk ringröta i potatis förekom på mellan 5 - 24% av gårdarna 2014. Dessa förekomster anses höga med tanke på hur betydelsefulla grödorna är. Odlarnas medvetenhet om åtgärder för att begränsa sjukdomarna var otillräckliga på grund av att det saknas information eller att den är otillgänglig. Odlingsmetoder såsom monokultur, samodling, större plantavstånd och genom att undvika att dela odlingsredskap visade ett tydligt samband med låg förekomst av mörk ringröta medan tät plantering, samodling och odling av bananer för ölbryggnings var kopplade till hög förekomst av xanthomonas-visnesjuka. Bakteriepopulationen av artkomplexet *R. solanacearum*, som orsakar mörk ringröta i potatis i Rwanda, domineras av stammar som ingår i fylogrupp II (*Ralstonia solanacearum* emend. Safni). Isolaten som användes för studier av mottagligheten hos olika potatis- och banansorter och närstående växtarter för bakterierna, *R. solanacearum* och *X. campestris* pv. *musacearum*, infekterade, vid inokulering, potatis respektive banan och deras nära släktingar. Alla inokulerade sorter var mottagliga men i något olika grad. Studierna av dessa bakteriella visnesjukdomar har ökat vår kunskap om dessa sjukdomar i Rwanda. De har också givit viktiga insikter om utveckling av, och informationsspridning om, hållbara odlingsstrategier för att begränsa sjukdomarna, vilket i sin tur kommer att förbättra livsmedels säkerheten i landet.

*Nyckelord:* bakteriesjukdomar, detektion, odlingssystem, spridningsvägar.

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# Dedication

**To:**

The Almighty God from whom all good things come  
My parents who watch me from heaven  
My loving family and friends

*The price of success is hard work, dedication to the job at hand, and the determination that whether we win or lose, we have applied the best of ourselves to the task at hand.*

Vince Lombardi



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## List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Uwamahoro F., Berlin A., Bucagu C., Bylund H., Yuen J. (2018). Potato bacterial wilt in Rwanda: occurrence, risk factors, farmers' knowledge and attitudes. *Food security*, 10, 1221-1235.
- II Uwamahoro F., Berlin A., Bylund H., Bucagu C., Yuen J. (2019). Management strategies for banana *Xanthomonas* wilt in Rwanda include mixing indigenous and improved cultivars. *Agronomy for Sustainable Development*, 39, 22
- III Uwamahoro F., Bucagu C., Berlin A., Bylund H., Yuen J. Host range and banana cultivars' susceptibility to *Xanthomonas campestris* pv. *musacearum* in Rwanda. (submitted)
- IV Uwamahoro F., Berlin A., Bucagu C., Bylund H., Yuen J. *Ralstonia solanacearum* causing potato bacterial wilt: Host range and cultivars' susceptibility in Rwanda. (submitted)
- V Uwamahoro F., Bylund H., Bucagu C., Berlin A. Population structure of the pathogen causing potato bacterial wilt in Rwanda. (manuscript)

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The contribution of Florence Uwamahoro to the papers included in this thesis was as follows:

- I Contributed to the study design, performed the survey and interviews, analysed data, wrote the paper manuscripts with the co-authors and corresponded with the journal.
- II Designed the study with the co-authors, collected data, analysed data, wrote the paper manuscripts with the co-authors and corresponded with the journal.
- III Contributed to the study design, performed field and lab works, data analysis, wrote the paper manuscripts with the co-authors and correspond with the journal.
- IV Contributed to the study design, performed field and lab works, data analysis, wrote the paper manuscripts with the co-authors and correspond with the journal.
- V Performed sampling and took part in laboratory works, data analysis and drafted the manuscript in collaboration with co-authors

## Abbreviations

AUDPC	Area Under Disease Progress Curve
CIP	International Potato Center
ECA	East and Central Africa
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	Food and Agriculture Organization Statistical Databases
GDP	Gross Domestic Product
MINAGRI	Ministry of Agriculture and Animal Resources
MINECOFIN	Ministry of Finance and Economic Planning
NGOs	Non-Government organizations
NISR	National Institute of Statistics Rwanda
PCR	Polymerase Chain Reaction
RAB	Rwanda Agriculture Board
RSSC	<i>Ralstonia solanacearum</i> species complex
USAID	United States Agency for International Development
<i>Xcm</i>	<i>Xanthomonas campestris</i> pv. <i>musacearum</i>



# 1 Introduction

## 1.1 Role of banana and potato in food security in relation to plant diseases

The world population keeps growing and is expected to reach 9.8 billion by the year 2050 (UN DESA, 2017) and this infers a growing demand for food in quality and quantity to feed this increasing population. The Food and Agricultural Organization of the United Nations (FAO) defines food security as: “When all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life.” (FAO, 2010a). Banana (*Musa* spp.) and potato (*Solanum tuberosum*) are important crops worldwide, as they contribute to food security, income generation, employment opportunities and economic development for the farmers (FAO, 2017).

Banana (dessert bananas, cooking bananas and plantains) ranks the sixth on the list of staple crops in the world, with a global production of around 86 million tons per year (FAO, 2017). Plantains and cooking bananas are staple foods, while dessert bananas are an important source of calories, minerals (such as potassium) and vitamins consumed as a fruit (Van Asten & Staver, 2012). The bananas that cannot be cooked or consumed as fruits are used for brewing beer (Karamura *et al.*, 2012). The banana’s ability to produce fruits all year round makes it an important food security and cash crop in the tropics (Van Asten & Staver, 2012). About one-third of bananas produced globally are grown in sub-Saharan Africa, where the crop provides more than 25% of food energy requirements for more than 100 million people (Tripathi *et al.*, 2009). In the East and Central Africa region, approximately 20 million people depend

on banana for food (Arias *et al.*, 2003). In countries like Uganda, Rwanda and Burundi, banana can account for as much as 60% of the daily caloric intake (Abele *et al.*, 2007).

Potato is the third most consumed food commodity worldwide after rice and wheat, and it has been recommended as a food security crop by the FAO (Devaux *et al.*, 2014). Potato provides more food much faster than any other major crop and is high in nutrient content including high content of carbohydrates, quality protein, and vitamins, especially vitamin C (Lutaladio & Castaldi, 2009; Prokop & Albert, 2008). In Africa, the area under potato production is about 1.89 million ha with an average yield of 13.2 t/ha (FAO, 2019). Rwanda is among the top five potato producing countries in Africa and the third in sub-Saharan Africa (FAOSTAT, 2015), which is noteworthy considering the land size of the country.

Pests and diseases can cause damage to crops, reduce the availability and access to food, increase the cost for food or result in changing traditional food preferences of peoples (FAO, 2017; Flood, 2010). Currently, about one billion people in the world are inadequately fed (FAO, 2017), and plant disease outbreaks aggravate the current deficit of food supply (Strange, 2006; Strange & Scott, 2005). Estimated losses of 20 to 40% in crop production annually are reported and improved global food security require solutions that address these losses and improving plant health (Savary *et al.*, 2011; Flood, 2010). Hence, protection of plants and crops against plant diseases has an evident role to meet the growing demand for food (Strange & Scott, 2005). The understanding of plant disease, the ability of the causing organism to infect host plants and the driving factors in a given area, provide the basis to suggest sustainable management approaches.

## 1.2 Agriculture in Rwanda - Possibilities and constraints

Rwanda is a small landlocked country in the East and Central Africa (ECA) with a total surface area of 26,338 km<sup>2</sup>. The country's population is about 12 million people, making Rwanda the most densely populated country in Sub-Saharan Africa with an average of 445 persons per km<sup>2</sup> (NISR, 2015). Agriculture is the backbone of the Rwandan economy (MINAGRI, 2011); it constitutes around a third of the country's gross domestic product (GDP) and about half of export earnings (NISR, 2015; MINECOFIN, 2013). Close to 70% of working individuals aged 16 years or above are usually employed in agriculture (NISR, 2018). Rwanda has three agricultural seasons based on the precipitation patterns: September of one year to February the following year (Season A); March to June of the same year (Season B); and July to September

of the same year (Season C) (NISR, 2016). The seasons A and B are the major cropping seasons. The crops grown in season C are dependent on irrigation in the marshland or in the highland areas where the rainfall is present throughout the year. Potato is the main crop produced during season C (NISR 2015).

Yet, the agriculture faces structural constraints, including rapid population growth and small size of agricultural lands (Giertz *et al.*, 2015). About 80% of land holdings in Rwanda are less than 1 hectare (ha), often divided into three to four plots, and over 70% of agricultural land is either on hills or on the side of hills (NISR, 2015). Agriculture in Rwanda is dominated by small-scale, subsistence farming under traditional agricultural practices and rain-fed agriculture. To address these challenges, the government of Rwanda launched the Crop Intensification Program in September 2007. The aim of the program is to increase agricultural productivity in high-potential food crops and ensure food security and self-sufficiency through an improvement of productive inputs use, irrigation coverage and soil quality (Kathiresan, 2011). Under this program, the six crops potato, maize, rice, wheat, cassava and beans were selected as priority crops.

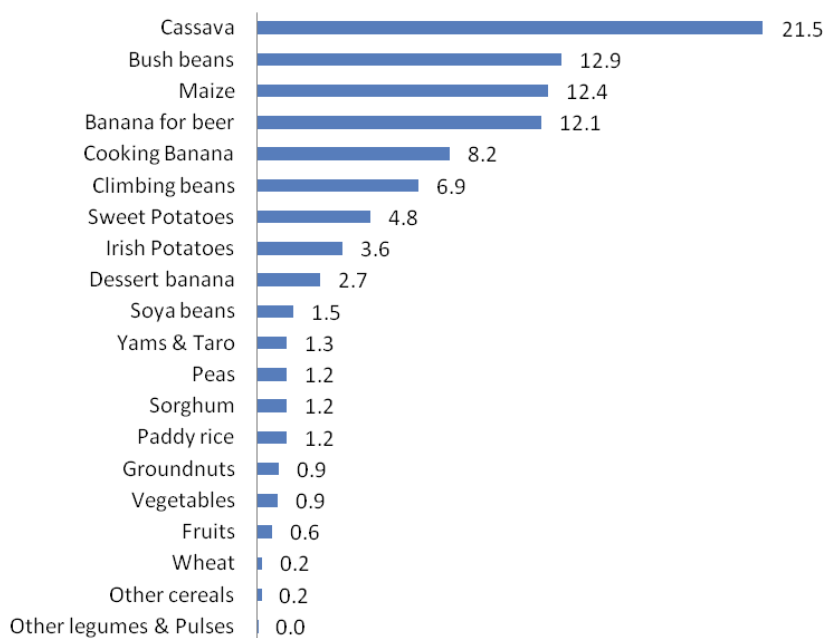


Figure 1. Share (%) of agricultural land by main crops in Rwanda (2014 Season A) (Seasonal Agricultural Survey by the National Institute of Statistics 2015).

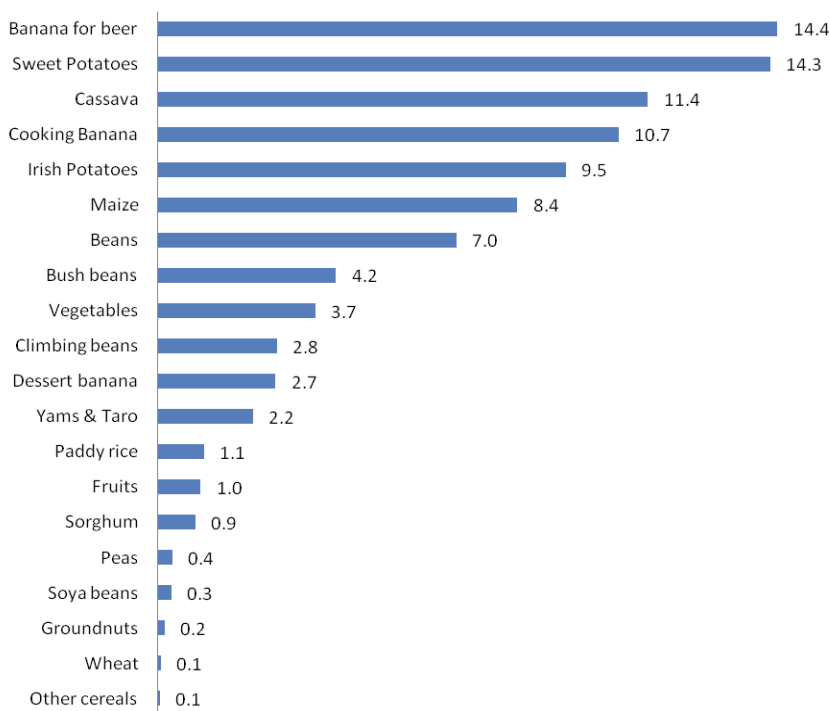


Figure 2. Share (%) of production by main crops in Rwanda (2014 Season A) (Seasonal Agricultural Survey by the National Institute of Statistics 2015).

Banana and potato are major crops in Rwanda and are grown year round. Together they constitute a large share in area cultivated and total production among other main crops (Figure 1 and Figure 2) (NISR, 2016). Nearly one quarter of Rwanda's arable land is allocated to banana (Mpyisi *et al.*, 2000). Banana production is favoured by the farmers because it assures continuous income under conditions of acute land shortage (Nsabimana *et al.*, 2008). The crop is grown for cooking, roasting, brewing, dessert and income generation (Nsabimana *et al.*, 2008; Gaidashova *et al.*, 2007). The Rwandan culture attaches considerable value to the banana crop, beyond economic gain. It is cultivated around the houses and is regarded as a symbol of prosperity and wellbeing (Lassoudière, 1989). Similarly, potato grow well in the whole country, from the highlands to the low and midlands (Munyemana & Oppen, 2000), with high productivity up to 30t/ha in the Northern and Western highlands where agricultural conditions are most favourable for potato production (MINAGRI, 2014). Potatoes have a high production potential per



area, can be stored relatively easily and, most importantly, can both be used as a staple food crop for household consumption and food security or sold as a cash crop (Cromme *et al.*, 2010). Rwanda is currently a major supplier of potato in the surrounding countries (MINAGRI, 2014).

However, the area harvested, yield and production of banana and potato in the country have been fluctuating over time. One example is the period between 2000 and 2017 (Table 1) (FAOSTAT, 2019), which could be attributed to a number of production constraints.

Table 1. *Area harvested, yield and production of Banana and Potato in Rwanda (2000-2017) (FAOSTAT 2019)*

Year	Banana			Potato		
	Area harvested (ha)	Yield (hg/ha)	Production (t)	Area harvested (ha)	Yield (hg/ha)	Production (t)
2000	360 470	61371	2212250	108983	87830	957202
2001	363 249	49114	1784058	117403	86222	1 012269
2002	358 863	77603	2784870	124972	83133	1038931
2003	360000	66884	2407837	133954	82084	1099549
2004	363383	67965	2469740	133418	80407	1072770
2005	361251	71781	2593080	135622	91740	1314050
2006	366294	72438	2653348	139043	77618	1275585
2007	380000	70684	2686000	124621	77618	967283
2008	370000	70378	2604000	127226	91329	1161943
2009	345414	86664	2993482	126167	102216	1289623
2010	333773	82366	2749152	150777	118679	1789404
2011	346411	87649	3036273	130000	90115	1171500
2012	349052	92235	3219465	120000	111475	1337700
2013	342694	96058	3291853	95000	130600	1240700
2014	322095	89646	2887440	62156	115678	719006
2015	317464	93886	2980546	75342	98567	742626
2016	322009	94344	3037962	106236	70718	751284
2017	464862	37197	1729150	93991	90028	846184

The biggest challenges to banana production in Rwanda and the East and Central Africa are posed by the declining soil fertility, lack of healthy seeds, pests and diseases (Nkuba *et al.*, 2015; Pemsl *et al.*, 2014; Karamura *et al.*, 2008; Gaidashova *et al.*, 2007). Locally grown banana cultivars in Rwanda are susceptible to fusarium wilt, nematodes, weevil borers and xanthomonas wilt diseases (Gaidashova *et al.*, 2007; Karamura & Tinzaara, 2007; Okech *et al.*, 2005). Correspondingly, major production constraints of potato in Rwanda consist of low soil fertility, unavailability of healthy seeds, poor market accessibility, shortage of financial investment, and lack of access to high yielding cultivars, limited land sizes, high production costs (for pesticides and seeds) and, pests and diseases (Mugabo *et al.*, 2018; Muhinyuza *et al.*, 2012).

The potato bacterial wilt, also known as southern bacterial wilt or potato brown rot (Agrios, 2005), cause more severe damages on potato crops than any other disease (Muhinyuza *et al.*, 2012).

## 1.3 Banana xanthomonas wilt disease

### 1.3.1 Background of the disease and the pathogen

Banana xanthomonas wilt is a devastating disease of banana in the East and Central Africa (Tripathi *et al.*, 2009), where it is confined (Blomme *et al.*, 2017a), but with high risk to spread further (Ocimati *et al.*, 2019). The disease is caused by the bacterium *Xanthomonas campestris* pv. *musacearum* (*Xcm*) (Yirgou & Bradbury, 1974). In 1968 the disease was first reported on the banana relative enset (*Ensete ventricosum*) and a few years later on banana in Ethiopia (Yirgou & Bradbury, 1974). The disease reached the Great Lakes region of East and Central Africa starting from central Uganda in 2001 (Tushemereirwe *et al.*, 2004), and from there it promptly spread to the Democratic Republic of Congo (DR Congo) (Ndungo *et al.*, 2006), Rwanda (Reeder *et al.*, 2007), Tanzania, Kenya and Burundi (Carter *et al.*, 2010). Banana xanthomonas wilt has many similarities to bacterial wilts of banana (moko, blood and bugtok diseases) that are caused by the *Ralstonia solanacearum* species complex (Blomme *et al.*, 2017a; Thwaites *et al.*, 2000). Once these pathogens have become established, they are difficult to control and impossible to eradicate (Eden-Green, 2004).

The pathogen *Xcm* attacks all banana cultivars and can result in up to 100% yield loss, especially in cropping systems where Musa ABB cultivars predominate (Nakato *et al.*, 2014). This severely affects food security, farm incomes and the environment (Nkuba *et al.*, 2015; Karamura *et al.*, 2010; Kagezi *et al.*, 2006). This has resulted in farmers abandoning the banana crop for alternatives such as cassava (Karamura & Tinzaara, 2007; Kalyebara *et al.*, 2006). The economic impact of the disease is substantiated by complete yield loss or reduced production and death of the mother plant and suckers, valuable in subsequent plant production cycles (Nakato *et al.*, 2018; Kubiriba *et al.*, 2012). The transmission pathways of *Xcm* include infected plant material, soil, cutting tools, long-distance trade, and vectors (Tinzaara *et al.*, 2011) (Figure 3). In addition, lack of knowledge among farmers on disease diagnosis and management, and cultural practices such as the use of unsterilized cutting tools, contribute to the rapid spread of *Xcm* (Mwangi & Nakato, 2009).

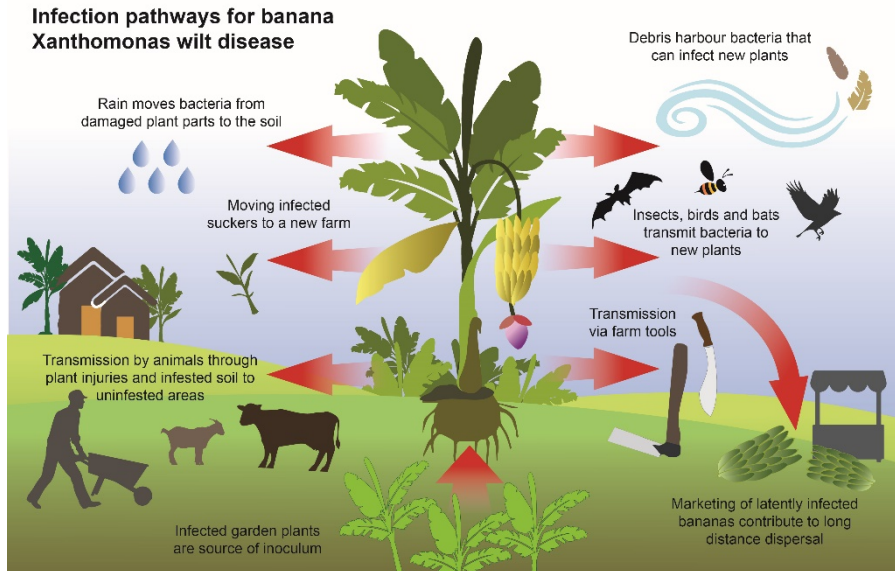


Figure 3. Transmission means of *Xanthomonas campestris* pv. *musacearum* causing banana xanthomonas wilt. The bacterium is spread by everything that gets in contact with an infected plant, such as insects and birds sucking nectar or feeding on ripen fruits, grazing animals, cutting tools and unintentional moving of infected soils on boots, symptomless infected suckers or marketed infected banana (**Paper II**).

### 1.3.2 History of banana xanthomonas wilt in Rwanda

In Rwanda, the disease was officially reported in 2005 in Rubavu district, western Rwanda (Reeder *et al.*, 2007), while the farmers in this area claimed to have noticed the symptoms around 2002-2003. It is probable that banana xanthomonas wilt entered Rwanda with latently infected bananas or planting materials due to continuous exchange of goods and people across the Rubavu-Goma border, since the first disease outbreaks in DR Congo were confirmed in 2001 near this border in the north of Goma (Reeder *et al.*, 2007; Ndungo *et al.*, 2006). Following the first report of the disease in Rubavu district during 2005, *Xcm* has progressively spread (Reeder *et al.*, 2007). In 2007, banana xanthomonas wilt was present in three districts namely Rubavu, Rutsiro and Rulindo (Karamura *et al.*, 2008; Muhinyuza & Gaidashova, 2006), and a few years later in 2009-2010 the disease was reported in 9 out of 12 surveyed districts (Night *et al.*, 2013). The disease affects the livelihoods of banana growing farmers and food security in the Rwanda and the East and Central Africa (Nkuba *et al.*, 2015).

### 1.3.3 Disease management

So far, there is no single method that has been proven to be effective in controlling banana xanthomonas wilt (Nakato *et al.*, 2018; Kubiriba & Tushemereirwe, 2014). Only cultural control practices are recommended as preventive measures for the containment of the disease (Rutikanga *et al.*, 2015; Karamura & Tinzaara, 2007) and these should be applied as a package (Kubiriba & Tushemereirwe, 2014). The disease could be managed if sources of inoculum are removed and opportunities for spread are reduced or eliminated (Blomme *et al.*, 2005; Eden-Green, 2004).

The recommended package of agricultural practices for the control of banana xanthomonas wilt include planting healthy suckers, cutting out the male buds with a forked stick, sterilizing farm tools and removal of infected plants (Nakato *et al.*, 2018; Blomme *et al.*, 2017b; Kubiriba & Tushemereirwe, 2014). Due to the associated high labor cost and the perceptions of low effectiveness of the disease management practices, some farmers do not stick to this recommendations and as a result, *Xcm* continues to spread (Jogo *et al.*, 2013; Rutikanga *et al.*, 2013). So far, no resistant cultivars have been identified (Kebede & Gemmeda, 2017; Tripathi & Tripathi, 2009; Ssekiwoko *et al.*, 2006b) and field observations in areas where the bacterium is present suggest that all commonly grown cultivars are susceptible. Furthermore, a number of crops and weeds have been shown to be alternative hosts to *Xcm* (Ocimati *et al.*, 2018; Chala *et al.*, 2016; Karamura *et al.*, 2015; Michael *et al.*, 2006; Ssekiwoko *et al.*, 2006a). These facts complicate the success in the banana xanthomonas wilt management.

## 1.4 Potato bacterial wilt

### 1.4.1 Background of the disease

Potato bacterial wilt has been estimated to affect about 1.7 million hectares in approximately 80 countries worldwide, with global damage estimates of over USD 950 million each year (Birch *et al.*, 2012). The disease is one of the major problem to the potato sector in developing countries and for farmers in sub-Saharan Africa and East and Southeast Asia, the control of potato bacterial wilt is among the priority research needs (Fuglie, 2007). There is no single method to control the disease effectively and all potato cultivars are vulnerable to the disease (Champoiseau *et al.*, 2010).

The disease is caused by strains of the *Ralstonia solanacearum* species complex (RSSC), one of the world's most important plant pathogenic bacterial

groups due to its lethality, persistence, wide host range, and broad geographical distribution (EPPO, 2018; Denny, 2007; Elphinstone, 2005) (Figure 4). The RSSC affects over 200 species from more than 50 botanical families globally (Pasicznik *et al.*, 2005), with the most important hosts belonging to the nightshade family (*Solanaceae*) (EPPO 2019; Hayward, 1994). Bacterial wilt symptoms on solanaceous crops appear as a sudden wilt (Agrios, 2005). Infected young plants die rapidly whereas older plants first show wilting of the youngest leaves, or one sided wilting and stunting, and finally the plants wilt permanently and die (Agrios, 2005). The vascular tissues of stems, roots, and tubers turn brown and in cross sections they ooze a whitish bacterial exudate (Priou *et al.*, 1999).

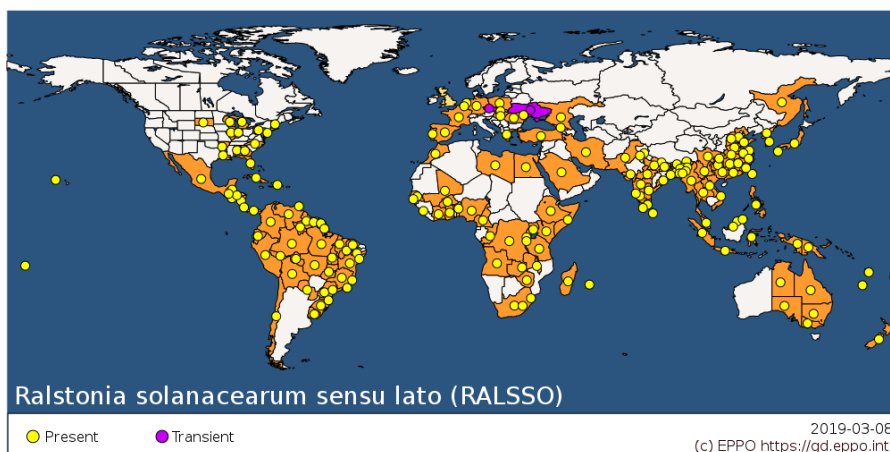


Figure 4. Geographical distribution of *Ralstonia solanacearum* species complex (EPPO 2019). Orange colour stands for countries or states where the bacterium presence was reported.

#### 1.4.2 History of the *Ralstonia solanacearum* species complex

The bacterium originally described as *R. solanacearum* was considered a ‘species complex’ due to significant variation at different levels (physiological, serological, genetic characteristics and host range) within the group (Fegan & Prior, 2006). In order to describe the intra-specific variability in within the RSSC, several systems of taxonomic classification have been proposed (Table 2). The name *R. solanacearum* was introduced by (Yabuuchi *et al.*, 1995). Traditionally, the bacterium was classified into five races on the basis of different host range (He *et al.*, 2011; Buddenhagen & Kelman, 1964) and six biovars according to the ability to oxidize three hexose alcohols and three disaccharides (Denny, 2007; Hayward, 1991). This old classification system is

nowadays inadequate because it is not predictive and some groups like race 1 contain very large variation (Patil *et al.*, 2012; Denny, 2007). Moreover, races and biovars are regarded to be informal groupings at the intra-sub specific level that are not governed by the code of nomenclature of bacteria (Patil *et al.*, 2012; Elphinstone, 2005).

There is no general correlation between races and biovars; except for biovar 2 strains that are almost always race 3 and vice versa. The race 3 strains were originally described as pathogenic on potato and tomato, but weakly virulent on other solanaceous crops (Buddenhagen & Kelman, 1964). Race 3 has sometimes been referred to as the “potato race” (French, 1994; Hayward, 1991; Buddenhagen & Kelman, 1964). The race 3 biovar 2 strains are known as cold tolerant strains in temperate climates and have been reported to cause potato bacterial wilt at high altitudes in East Africa including Kenya, Rwanda, and Ethiopia (Kassa, 2016; Muthoni *et al.*, 2013a; He *et al.*, 2012). These strains, originated from the Andes, have also spread through the tropical highlands and subtropical warm-temperate areas throughout the world. The RSSC is considered a quarantine pathogen in Europe (Health *et al.*, 2019; Janse *et al.*, 2004). The strains in race 3 biovar 2 are considered select agents in the United States under the Agricultural Bioterrorism Protection Act (Hawks, 2002).

Table 2. *Different taxonomic changes for the RSSC (adapted from EPPO 2017/194)*

Source	Taxonomic revision			
Smith 1896	<i>Bacterium solanacearum</i>			
Smith 1914	<i>Pseudomonas solanacearum</i>			
Yabuuchi <i>et al.</i> , 1992	<i>Burkholderia solanacearum</i>			
Yabuuchi <i>et al.</i> 1995	<i>Ralstonia solanacearum</i>			
Fegan and Prior 2005	<i>Ralstonia solanacearum</i> species complex			
	Phylotype I	Phlotype II	Phylotype III	Phylotype IV
Safni <i>et al.</i> , 2014	<i>R.</i> <i>pseudosolanacearum</i>	<i>R.</i> <i>solanacearum</i>	<i>R.</i> <i>pseudosolanacearum</i>	<i>R. syzygii</i>

The term "species complex" was first introduced by Gillings (1994). Based on phylogenetic relationships, the classifications of the RSSC have evolved into four distinct phlotypes (I-IV) in relation with their geographical origin and further subdivided into distinct sequevars (Fegan & Prior, 2005); phylotype I originating from Asia, phylotype II from the Americas and Caribbean, phylotype III from Africa, and phylotype IV from Indonesia, Australia, and Japan (Wicker *et al.*, 2012; Fegan & Prior, 2005). The strains in phylotype II sequevar 1 and phylotype II sequevar 2 (known historically and for quarantine purposes as race 3 biovar 2 or R3bv2) are responsible of potato

brown rot or bacterial wilt (Cellier & Prior, 2010). Safni *et al.* (2014) regrouped the RSSC into three species, i.e. *R. solanacearum* that corresponds with phylotype II, *R. pseudosolanacearum* that corresponds with phylotypes I and III and *R. syzygii* (subsp. *celebensis* and *indonesiensis*) equivalent with phylotype IV strains, which include the blood disease bacterium (BDB). This new reclassification of RSSC was confirmed by combining genomics, proteomics and functional phenotypic assays by Prior *et al.* (2016). Here we use the term *R. solanacearum* to designate the species that coincide with phylotype II (Safni *et al.*, 2014). The relationship between classification of *R. solanacearum* based on races, biovars, phylotypes, sequevars and type of disease caused by each class are summarized in Table 3 adapted from García *et al.* (2019).

Table 3. Current *infra specific classification of the RSSC* (García *et al.*, 2019)

Phylotype	Sequevar	Biovars	Ecotype*	Races	Species
I	12, 14, 16, 18	3, 4, 5	BW of <i>Solanaceae</i> , ginger, mulberry	1,4,5	<i>R.</i> <i>pseudosolanacearum</i>
IIA	1, 2, 3, 4, 5, 6, 7, CIP 10, CIP223, NCPBP39	2-T, 1, 2	BW of <i>Solanaceae</i> , <i>Musa</i> spp.	1,2,3	<i>R. solanacearum</i>
IIB	1, 2, 3, 4, 5, 6, 7, CIP 10, CIP223, NCPBP39	2-T, 1, 3	Moko diseases, NPB, brown rot of potato, BW of tomato and geranium	1,2,4	<i>R. solanacearum</i>
III	19, 20, 21, 22, 23	2-T, 1	BW of <i>Solanaceae</i>	1	<i>R.</i> <i>pseudosolanacearum</i>
IV	8, 9, 10, 11	2-T, 1, 2	BW of <i>Solanaceae</i> , BDB of <i>Musa</i> spp., Sumatra disease of clove	1	<i>R. syzygii</i>

\*BW: bacterial wilt, NPB: non-pathogenic on banana, these are new pathogenic variants virulent on *Anthurium*, *Heliconia* and cucurbits but not on banana, BDB: blood disease of banana.

Like almost all bacterial plant pathogens, the RSSC enters into plants via wounds made by tools during post emergence cultivation or by nematodes and insects in the soil and natural openings. Once inside the plant, the bacteria move towards the vascular bundles to finally colonize the xylem (Poussier *et al.*, 2003). Inside the xylem, the bacteria multiply and block the vascular vessels causing a water shortage throughout the plant. This causes the plant to wilt and eventually die (Poussier *et al.*, 2003). The RSSC has an exceptional ability to survive for long periods in free water, soil and the rhizosphere of non-hosts or latently infected hosts (Hayward, 1991). Figure 5 illustrates the means of survival and spread for the RSSC in the potato cropping systems. The bacterium can survive in the soil for a few months to a few years, and plant debris or rotting tubers help the pathogen to survive from season to season in

the absence of host crops (Van Elsas *et al.*, 2000). From the soil RSSC enters plant roots, invades xylem vessels and spreads rapidly to aerial parts of the plant through the vascular system where its high level of multiplication leads to wilting symptoms and, ultimately, plant death (Genin, 2010). The ability of RSSC to survive in soils for many years and to form latent infections within indigenous weeds contributes to the difficulties of eradication of the bacterium (Huet, 2014; Wenneker *et al.*, 1999; Hayward, 1991).

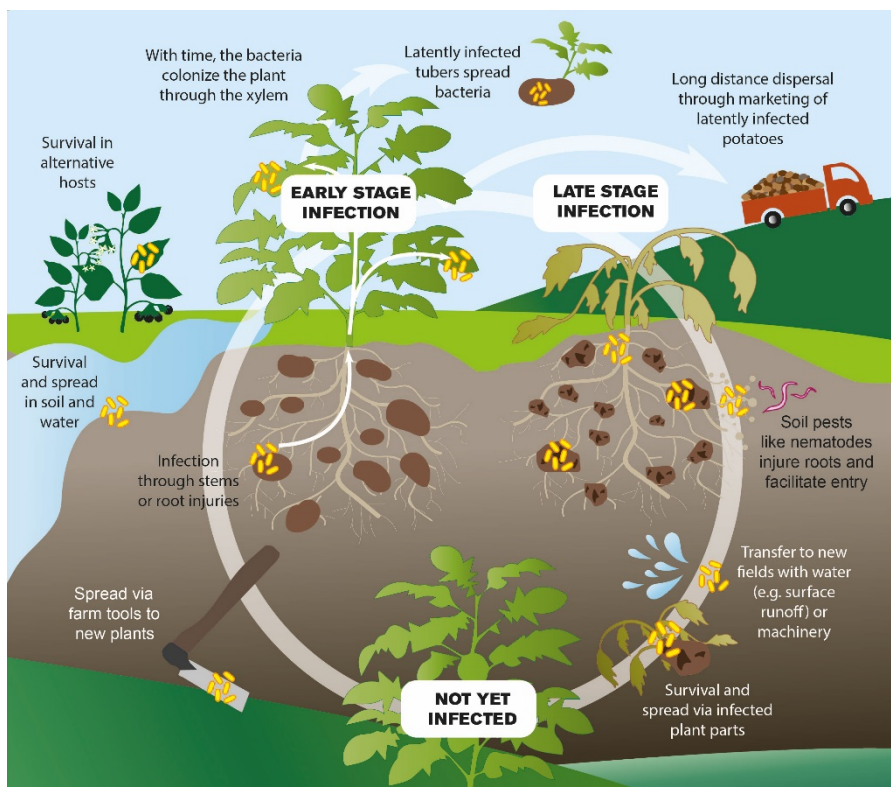


Figure 5. The means of survival and spread for the bacterium *Ralstonia solanacearum* species complex in potato.

### 1.4.3 Potato bacterial wilt in Rwanda

There is limited information on potato bacterial wilt disease history in Rwanda, since the exact time that the disease appeared in the country is not documented. However, studies conducted in the 1980's reported bacterial wilt as the second most important disease of potato production in Rwanda after late blight (caused by *Phytophthora infestans*) (Scott, 1988; Devaux *et al.*, 1987; Bicamumpaka & Devaux, 1984). These reports indicate that the disease must have reached



Rwanda before or in the early 1980's. Muhinyuza *et al.* (2012) also reported bacterial wilt as important disease of potato next to late blight disease and that, according to the farmers, bacterial wilt cause more severe damage than those caused by late blight. Scott (1988) stressed that the disease was more severe in the southern and eastern Rwanda. Recently, a study on virulence and characterization of isolates of potato bacterial wilt caused by *Ralstonia solanacearum* (Smith) in Rwanda revealed the presence of biovar 3 (Mutimawurugo *et al.*, 2019). The knowledge on the current disease distribution, the responsible factors and diversity in the population structure is still lacking. To be able to suggest a disease management strategy, a good understanding about the disease and the population structure of the causing pathogen is essential.

#### 1.4.4 Management of potato bacterial wilt

The management of potato bacterial wilt caused by RSSC is difficult because it is a soil borne pathogen, has wide host range, long survival in the soil, and has wide biological variation (Martin & French, 1985). No single control method has been found to be 100% effective, although in locations where the pathogen is established, some level of bacterial wilt control has been possible through use of a combination of diverse methods including phytosanitation and cultural practices, chemical control, biological control, and host resistance (EPPO, 2018; Muthoni *et al.*, 2012; Champoiseau *et al.*, 2010). Once a potato plant or tuber is infected with bacterial wilt, there is no guaranteed cure, emphasizing that control measures should be applied to prevent infection.

Phytosanitation practices, like planting healthy seeds in uninfested soils and quarantine measures, have been effective to control bacterial wilt in places where bacterial wilt is endemic, or in locations where it is present but not yet established (Muthoni *et al.*, 2012; Champoiseau *et al.*, 2010). A number of cultural practices consisting of crop rotation, intercropping, delayed planting, soil amendments, positive selection, and negative selection have been recommended for the management of potato bacterial wilt (Gildemacher *et al.*, 2012; Champoiseau *et al.*, 2010; Lemaga *et al.*, 2005; Priou *et al.*, 1999).

Most potato cultivars are susceptible to RSSC but some cultivars are tolerant at least in some regions (Muthoni *et al.*, 2014; Felix *et al.*, 2011; Ateka *et al.*, 2001a). Seven potato genotypes from two wild Andean potato species were identified to have high levels of resistance to bacterial wilt and tuber infection in recent years, which provide new resistance sources for developing commercial resistant potato cultivars (Salgon *et al.*, 2017; Patil *et al.*, 2012; Champoiseau *et al.*, 2010). Another possible source of resistance is eggplant,

since several studies have demonstrated resistance to bacterial wilt in eggplant (Salgon *et al.*, 2017; Gopalakrishnan *et al.*, 2014; Lebeau *et al.*, 2011). Other alternative control measures have also been investigated. A number of soil bacteria and plant growth promoting rhizobacteria have been investigated for their potential role in the control of potato bacterial wilt (Champoiseau *et al.*, 2010), however, none is currently available commercially (Muthoni *et al.*, 2012). Chemical control by fumigating contaminated soils with methyl bromide was an option to control race 3 biovar 2 (Champoiseau *et al.*, 2010), but this is a very expensive and laborious control method and cannot be used on large areas. Moreover, the use of chemicals like sodium hypochlorite is recommended for disinfecting farm tools (Sharma, 2017).

## 1.5 Justification of the study

Agriculture is the main economic activity in Rwanda with 66% of the population engaged in the sector (NISR 2018). Banana and potato dominates crop production in the country and hence contribute to the livelihood and food security of the farmers (NISR 2016). However, these crops are threatened by diseases, the most worrying being xanthomonas wilt in banana and bacterial wilt in potato. For both banana xanthomonas wilt caused by *Xanthomonas campestris* pv. *musacearum* (*Xcm*) and potato bacterial wilt caused by RSSC, there is no single method to control the diseases effectively, resulting in total yield losses if management strategies are not implemented properly. None of the available banana or potato cultivars is resistant to the *Xcm* and RSSC respectively. These bacteria have similar means of transmission and management of their respective diseases rely on agricultural practices (Nakato *et al.*, 2018; Mansfield *et al.*, 2012; Saddler, 2005).

For the proper management of any kind of disease, a detailed study on the status of the disease, the causal organism, mode of spread and risk factors associated with that disease in a given area is of paramount importance. The reports of potato bacterial wilt occurrence in Rwanda are dated (French, 1994; Devaux *et al.*, 1987; Bicumupaka & Devaux, 1984), and should not constitute the sole base for future management plans. Similarly, *Xcm* causing banana xanthomonas wilt spread rapidly in the country since the first report in Rwanda (Night *et al.*, 2013; Reeder *et al.*, 2007). Hence continuous assessment of the disease progress and identification of factors responsible for the current occurrence will help stakeholders in the banana value chain to plan and implement sustainable management strategies. The information that an

individual has about a new technology forms the basis of the perceptions and attitudes this individual develops towards the technology (Adam *et al.*, 2015; Meijer *et al.*, 2015). Understanding the farmers' awareness on the disease detection and spread of the disease causing pathogens, and their attitudes towards the disease management will help to improve extension services.

Moreover, the source of information that the farmers use in these aspects will provide insight as to why and how extension services can be improved to better suit the receivers. Knowledge about host range to these bacteria in Rwandan farming systems provides the understanding about other crops that may harbor the bacteria and should not be rotated or intercropped with potatoes and banana. Similarly, the knowledge about level of susceptibility among commonly grown cultivars is important for disease management and control. Last but not least, the population structure of the RSSC causing potato bacterial wilt in Rwanda is not yet known and this is important to understand disease patterns and spread.



## 2 Objectives of the thesis

The main aim of this thesis is to improve the understanding of two of the most damaging diseases in Rwandan crop production to limit their negative effect on food security and economic situation for farmers. This was achieved by comparing the biology, risk factors, and farmers' awareness of banana xanthomonas wilt and potato bacterial wilt and how these affect the occurrence of the diseases in Rwanda. The underlying hypothesis is that the understanding of biological and epidemiological aspects related to the banana xanthomonas wilt and potato bacterial wilt diseases and their causing pathogens will help to improve management strategies for these diseases. The specific objectives are:

- To assess the risk factors associated with the occurrence of banana xanthomonas wilt and potato bacterial wilt (**paper I and II**).
- To investigate if crops intercropped or rotated with banana or potato, crops in the same family and crops grown in the neighboring fields to banana and potato, are susceptible to *Xcm* from banana or RSSC from potato respectively (**paper III and IV**).
- To assess the susceptibility level of commonly grown banana and potato cultivars to *Xcm* and RSSC respectively (**paper III and IV**).
- To investigate the population structure of RSSC causing potato bacterial wilt in Rwanda (**paper V**).



## 3 Materials and Methods

### 3.1 Field surveys and interviews

To determine the factors associated with the occurrence of banana xanthomonas wilt and potato bacterial wilt in Rwanda, two separate surveys were conducted in 120 banana farms and 120 potato farms from eight different districts during 2015, and in 150 banana farms from ten different districts during 2016. The owners of the farms were interviewed and disease surveys were concurrently conducted in one of their fields (**paper I** and **II**). Both major and minor banana and potato growing districts respectively were included in these studies. The same sampling structure and questionnaire design was used for both studies, with minor adjustment depending on the crops specifications. It was assumed that the prevalence of banana xanthomonas wilt and potato bacterial wilt in Rwanda is influenced by many factors including farm location, cultural practices, disease awareness by the farmers, management practices and source of information in these aspects.

It is already known that the disease causing bacteria *Xcm* and the RSSC are highly transmissible (Buregyeya *et al.*, 2014; Tinzaara *et al.*, 2016; Adikini *et al.*, 2013; Muthoni *et al.*, 2013a), and that everyday farming activities can spread these bacteria unintentionally (Jogo *et al.*, 2013; Mwangi *et al.*, 2008; Denny, 2007). Understanding these factors will help the decision makers and stakeholders in the value chain of potato and banana on how to improve the disease management efforts and information materials to the farmers. In each surveyed farm, the nearest banana or potato field to the owner's home was surveyed for banana xanthomonas wilt or potato bacterial wilt respectively. The diseases were identified in the field based on visual symptoms as previously described (Tripathi *et al.*, 2009; Agrios, 2005). The incidence (percentage of infected plants over the total number of surveyed plants) and

severity (extent of disease symptoms) were recorded similarly for both diseases except that banana xanthomonas wilt incidence was calculated on a banana mat instead of an individual plant. The severity for each of the surveyed potato plants or banana mats was recorded. During the field surveys in 2015, crops and plant species grown in or around potato and banana fields were recorded to be used in the study of *Xcm* and RSSC host range in the greenhouse (**paper III** and **IV**).

We used logistic regression to assess the relationship between diseases occurrence (incidence and severity) with the independent variables as defined by (Everitt & Hothorn, 2006). A total of thirteen independent variables were tested in association with banana xanthomonas wilt occurrence (**paper II**) and six variables tested with potato bacterial wilt occurrence (**paper I**). Logistic regression calculates the probability of a given binary outcome (response) as a function of the independent variables (Everitt & Hothorn, 2006). Hence, the dependent variables (incidence or severity) were classified into distinct categories of binomial qualitative data. The class boundaries, that divide the groups into approximately equal totals, were chosen, thus, yielding a binary dependent variable. The test was done in a single, multiple and reduced models as described in (Fininsa & Yuen, 2001). For both diseases, the mean comparisons of incidence and severity between districts were calculated using a one way ANOVA and multiple comparisons of the least significant difference (LSD) (**paper I** and **II**).

### 3.2 Host range of *Xcm* and RSSC

A total of 14 plant species recorded in the vicinity of potato plants and 17 plant species in the neighborhood of banana plants were used to assess the host range of *Xcm* and the RSSC in Rwanda. Potato and banana were used as positive controls. We assumed that crops intercropped or rotated with banana or potato, plants in the same family and plants grown in the neighboring fields to banana and potato can host the RSSC infecting potatoes or *Xcm* infecting banana. To assess that, we assembled those test plant species in the greenhouse and allowed them to establish prior to inoculation (Figure 6a; 6b).





**Figure 6.** The experimental set up of *Xcm* and the RSSC host range study. (a) Test plants for *Xcm* host range, (b) test plants for RSSC host range; (c) inoculating test plants with the bacteria; (d) observation of symptoms. Photos: F. Uwamahoro.

The samples of *Xcm* and the RSSC bacteria were isolated from symptomatic banana and potato samples collected in the neighborhood of the study sites (Musanze, 1°33'16.2"S 29°38'26.2"E), tested for virulence ability in the known hosts respectively and the bacteria were again re-isolated for further use. The isolated bacteria were then injected to healthy plantlets of test plant species by using the hypodermic syringe under sterile conditions (Figure 6c). Control plantlets of each test plant species were inoculated with sterilized water. All the experimental plants were observed for disease symptoms expression (Figure 6d). Parameters including time to first symptoms, time to complete wilting, area under disease progress curve (AUDPC), bacterial ooze test and Polymerase Chain Reaction (PCR) helped to rate test plant species as hosts or non-hosts to *Xcm* (**paper III**) or to the RSSC isolates in this study (**paper IV**).

### 3.3 Screening cultivars for *Xcm* and RSSC susceptibility

Five banana cultivars and nine potato cultivars under multiplication in the public tissue culture laboratories of Rwanda were used to identify the susceptibility level of banana cultivars to *Xcm* causing banana xanthomonas wilt and the susceptibility of potato cultivars to potato bacterial wilt (**paper IV** and **III**). It was assumed that potato and banana cultivars currently multiplied by the public tissue culture laboratories are pathogen free. Inoculation procedures and disease assessments were performed in a manner similar to the host range studies.

In **paper III** and **IV**, the average percent wilt incidence and severity data of symptomatic plant species and test cultivars were used to calculate the area under disease progress curve (AUDPC). The variation in days to first symptoms and to complete wilting among test plants and cultivars were tested using the Kaplan-Meier curves and Cox proportional hazard in the R package OIsurv (Diez & Diez, 2013; Nesi *et al.*, 2013; Rich *et al.*, 2010).

### 3.4 Population structure of the *R. solanacearum* species complex

To investigate the structure of RSSC strains causing potato bacterial wilt in Rwanda (**paper V**), infected potato samples were collected during 2014 and 2016 in the eight districts surveyed for potato bacterial wilt in **paper I**. The ooze from collected samples were squeezed on to Whatman™ FTA™ cards (GE Healthcare) for DNA extraction and PCR tests. The phylotype of each of the DNA samples was identified by using four phylotypes specific primers to RSSC in a multiplex PCR reaction (Fegan & Prior, 2005). Subsequently, the population structure and diversity was investigated by using microsatellites primers previously described (Ravelomanantsoa *et al.*, 2018; N'Guessan *et al.*, 2013) (**paper V**).

The genotypic richness was reported as number of samples and genotypes. Based on clone corrected datasets, the genotypic diversity was calculated as Shannon-Weiner index and Simpson's index (Shannon 2001, Simpson 1949). To evaluate the population structure, a principal component analysis was performed based on the genetic differences between each group of samples and minimum spanning networks based on Bruvo's distance (Bruvo 2004).

## 4 Risk factors associated with banana xanthomonas wilt and potato bacterial wilt occurrence

The first objective of this thesis was to investigate the occurrence of banana xanthomonas wilt and potato bacterial wilt diseases and to identify the influence of factors such as location, cultural practices, and farmers' knowledge and attitudes toward the diseases in Rwanda. Understanding the association of different risk factors with disease occurrence would help to identify the most important risks and focus efforts on developing an integrated and sustainable management package (Rusuku *et al.*, 1997). Regarding the rapid spread of banana xanthomonas wilt in the East and Central Africa, continuous assessment of the disease occurrence in each country and at regional level is required to identify the extent of disease spread. Similarly, the reports of potato bacterial wilt occurrence in Rwanda are not recent and up-to-date information about the disease is required.

The information about risk factors associated with these diseases and the level of awareness by the farmers in Rwanda is currently lacking or inadequate. In public and crop health epidemiology, the relationships between individual persons' ways of life and their associated health risk problems have been demonstrated (Savary *et al.*, 2011; Titus-Ernstoff *et al.*, 2002). Predictions of these risk factors help to prevent the disease and to guide research prioritization (Savary *et al.*, 2011). The acquisition of knowledge by farmers is the basis of the decision-making process regarding technological adoption, which in turn determines the perceptions and attitudes this individual develops towards the technology (Meijer *et al.*, 2015).

## 4.1 Diseases occurrence

The results in **paper I** showed the presence of potato bacterial wilt in all the surveyed districts (Table 4), and higher disease records in areas where potato is considered a minor crop. On the other hand, banana xanthomonas wilt was also present in all surveyed districts but higher in major banana growing areas (Figure 7) (**paper II**).

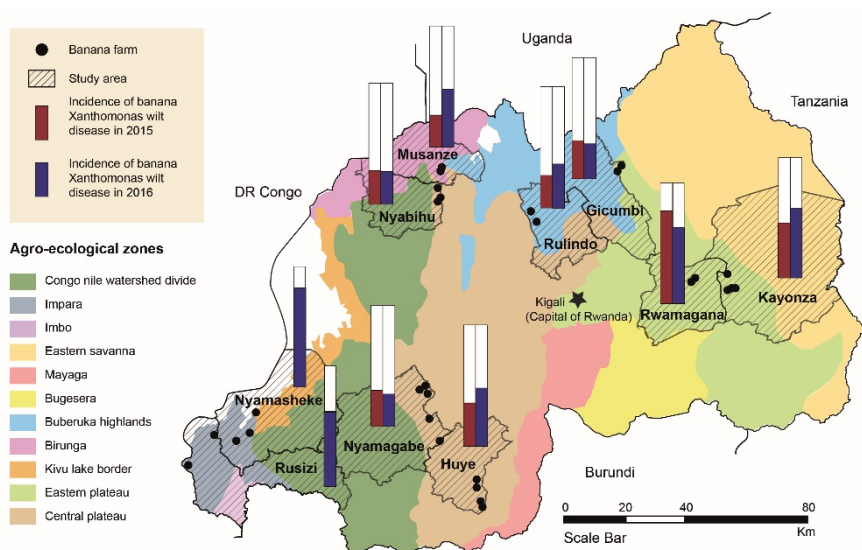
Potato bacterial wilt occurrence and widespread distribution in all study districts may be attributed to the longstanding disease presence in the country (Van der Zaag, 1985), and to informal seed system and the uncontrolled movement and exchange of potatoes locally (USAID, 2016). The higher disease occurrence in minor potato growing districts reflects the lack of extension services because the crop is not a priority in these areas and the biophysical factors are not optimal for potatoes (Verdoodt & Van Ranst, 2006). Potato has a short cropping cycle, thus it is continuously grown to secure food in the middle of other major crops' growing periods. However, growing potatoes continuously in infested soils facilitate the multiplication of the bacteria and hence increase disease incidence and severity (Muthoni *et al.*, 2013b).

Table 4. *Potato bacterial wilt disease incidence and severity in the surveyed districts of Rwanda during 2015. Major potato growing districts are Gicumbi, Musanze, Nyabihu and Nyamagabe. Means that are followed by the same letters are not significantly different in incidence and severity ( $p < 0.05$ )*

Districts	Incidence (%)	Severity (%)
Kayonza	24.4 <sup>a</sup>	13.1 <sup>a</sup>
Huye	16.7 <sup>b</sup>	8.1 <sup>bc</sup>
Rwamagana	16.6 <sup>b</sup>	9.9 <sup>b</sup>
Rulindo	14.1 <sup>bc</sup>	8.7 <sup>b</sup>
Nyabihu	8.6 <sup>cd</sup>	5.2 <sup>cd</sup>
Musanze	8.5 <sup>d</sup>	5.0 <sup>cd</sup>
Nyamagabe	6.1 <sup>d</sup>	3.7 <sup>d</sup>
Gicumbi	5.2 <sup>d</sup>	3.1 <sup>d</sup>

In **paper II**, we found that the occurrence of banana xanthomonas wilt have increased and spread to new places compared to the previous report from Rwanda (Night *et al.*, 2013), that was also observed in the surrounding countries (Shimwela *et al.*, 2016). This can be attributed to inappropriate application of management practices by the farmers and failure in applying regulations and quarantine measures (Tinzaara *et al.*, 2011). In addition, some farmers in the East and Central Africa region have been reluctant to apply the laborious recommended practices, because they expect immediate results from implementation of the disease control measures which is sometime not likely to

be, hence the disease continued to spread (Tinzaara *et al.*, 2011; Karamura *et al.*, 2010).



*Figure 7.* Agro- ecological zones of Rwanda based on differences in climatic, topographic and edaphic environment (Verdoodt & Van Ranst, 2006) and banana xanthomonas wilt distribution in the surveyed districts in 2015 and 2016. The disease was present in all surveyed districts. Black dots indicate some surveyed farms and bars show xanthomonas wilt incidence in 2015 and 2016 (**paper II**).

The reports of banana xanthomonas wilt and potato bacterial wilt in this study highlight the need to improve extension approaches and to support early detection of the diseases. Speeding up the exchange of adequate knowledge about the disease in order to make quick and appropriate decisions about control strategies, before the disease reaches the economic threshold level, will help to reduce the impact of these diseases. It is also crucial to increase the level of farmers' knowledge to understand the recommended management strategies for potato bacterial wilt and banana xanthomonas wilt, as well as the ability to afford and implement these management strategies.

## 4.2 Relationship of the diseases with farm locations and farming practices

In each of the surveyed farms for banana xanthomonas wilt and potato bacterial wilt, farming practices and farm locations were recorded (**paper II**

and I). In **paper II**, the occurrence of banana xanthomonas wilt was tested in association with a number of variables in both single and multiple models. Only agro-ecological zone, spacing, banana type and cropping systems were significantly ( $p<0.05$ ) associated with banana xanthomonas wilt incidence, whereas the agro-ecological zone, cropping system, banana type and cultivars were significantly ( $p<0.05$ ) associated with the severity in single and multiple models. Similarly, in **paper I**, six variables were tested in association with potato bacterial wilt incidence and severity in the single and multiple models. The results showed significant association ( $p<0.05$ ) of potato bacterial wilt incidence and severity with altitude, cropping systems, spacing (crop density) and sharing farm tools when entered first and last into the model.

High incidence of banana xanthomonas wilt was positively associated with major banana growing zones, intercropping system, brewing banana type, dense and moderate spacing. Similarly, higher banana xanthomonas wilt severity was associated with major banana growing zones, intercropping systems, brewing banana type and homogenous cultivation of either improved or indigenous cultivars (**Paper II**). High potato bacterial wilt incidence and severity showed high probability of association with farm tools sharing while low incidence and severity of potato bacterial wilt were highly associated with high altitude, rotation and intercropping system as well as wide spacing between plants (**Paper I**).

Our results demonstrate that incidence and severity of potato bacterial wilt in the surveyed districts could be reduced by applying management methods such as low plant density, intercropping, crop rotation and avoidance of sharing farm tools practices (**paper I**). Moreover, future banana xanthomonas wilt management extension efforts should take into consideration the effects of practices like intercropping, dense spacing, and homogeneity in grown cultivars on high disease occurrence and the agro-ecological zones in high risk of disease (**paper II**). These findings show the urgency of improving extension services with updated practices and reinforcing disease monitoring efforts in order to stop new infections and further spread of the potato bacterial wilt and banana xanthomonas wilt causing bacteria in Rwanda.

#### 4.3 The farmers' awareness, management attitudes and source of knowledge

The lack of knowledge on disease recognition and management is considered a major factor to the rapid spread of *Xcm* (Mwangi & Nakato, 2009). The farmers' perceptions on banana xanthomonas wilt or potato bacterial wilt were

assessed by using face to face interviews with the farmers whose farms were surveyed for disease occurrence. Farmers had the opportunity to mention more than one method they know or apply for detection, spread and management for banana xanthomonas wilt of potato bacterial wilt, as well as the source of this information (**paper I and II**). It was also previously demonstrated that the accuracy of information that the farmers determines the willingness to try using it (Adam *et al.*, 2015). To assess the farmers' source of knowledge on banana xanthomonas wilt or potato bacterial wilt, a list of 15 possible sources was provided to the interviewed farmers so that they could indicate the sources where they retrieved the information on the diseases under study (**paper I and II**).

The majority of banana growing farmers in this study could detect banana xanthomonas wilt disease by the wilting leaves and premature ripening of the fruits (**paper II**), as observed in Night *et al.* (2013). Similarly many potato growing farmers could detect potato bacterial wilt mainly based on wilting plants and rotting tubers (**paper I**). However, the knowledge of disease spread and management was limited in all the districts. Inadequate knowledge by the farmers in this study could be explained by inaccessibility to the accurate information since most of the farmers had received information about the diseases mainly from their fellow farmers and relatives (**paper I and II**). It is essential to improve the information source and communication channels to ensure that the correct information reaches the farmer. In addition, there was inconsistency between awareness and the application of disease management strategies by the farmers in this study. Hence, there is a need to understand the risk attitudes of farmers in relationship to disease management methods and for policy makers to improve extension services and extension messages accordingly.





## 5 Host range of *X. campestris* pv. *musacearum* and RSSC

These studies were designed to verify the ability of the bacteria *Xcm* and the RSSC to infect crops intercropped and rotated with banana or potato, crops grown in neighboring fields to banana or potato and plant species in the same family with banana or potato in Rwanda (**paper III** and **IV**). Understanding the hosts of these bacteria, in the banana and potato farming systems in Rwanda, helps to advise farmers on how to limit the inoculum sources and spread of the pathogens.

### 5.1 Hosts of *X. campestris* pv. *musacearum* in Rwanda

Among the eighteen plant species included in the study to assess the host range of *Xcm*, the bacterium infected only banana and banana relatives including wild banana, enset, and *Canna* species (Table 5) (**paper III**). The symptomatic plants showed differential sensitivity to the *Xcm* infestation. This has been demonstrated by high significant difference ( $p < 0.0001$ ) in days to first symptom expression and days to complete wilting among symptomatic plants. The ooze test also confirmed the presence of the bacterium in the symptomatic plant species. Our results confirmed previous findings that *Xcm* infecting banana, could also cause disease on enset, wild bananas and *Canna* spp. (Karamura *et al.*, 2015; Ssekiwoko *et al.*, 2006a). However, the *Xcm* isolate used in this study could not infect sorghum and maize, thus, contradicting earlier studies (Chala *et al.*, 2016; Aritua *et al.*, 2008). Concurrently, PCR tests using *Xcm* specific primers identified the bacterium in symptomatic hosts and some other plant species that were symptomless in the inoculation study, including maize and sorghum (Table 5). Factors like isolate type, host cultivar or different growing conditions may influence the expression of symptoms.

The recovery of *Xcm* by specific primers used on samples from symptomless crops (groundnuts, maize, pumpkins and sorghum) indicates that even if these crops did not express symptoms, they can harbor the bacterium (Ocimati *et al.*, 2018; **paper III**). Hence, these crops that are often

intercropped with banana or grown in neighboring fields to banana, can contribute to *Xcm* dissemination if contaminated. Our results agree with previous studies that could not observe the symptoms on maize, but re-isolated the bacterium from inoculated maize and hence confirmed the ability of maize to harbor the bacteria (Ocimati *et al.*, 2018; Karamura *et al.*, 2015).

Inoculated banana and enset plants showed higher values of AUDPC compared to *Canna* species and wild banana. Similarly, the days to symptoms expression and days to complete wilting showed that banana and enset plantlets were the first to show the symptoms and to wilt completely, while *Canna* species were the last. Enset and banana are the initial hosts to the bacterium which could be the reason that they showed the symptoms earlier than other host plant species (Yirgou & Bradbury, 1974; Yirgou & Bradbury, 1968). Disease management education material to banana farmers should also include the avoidance of cultivating plants susceptible to *Xcm* in close proximity to banana plants or fields.

Table 5. Observation of symptoms in the greenhouse, ooze streaming and PCR tests for *Xcm* presence or absence in the inoculated plant species. (+) denotes observation of symptoms in the greenhouse, bacterial streaming in a glass of water and positive PCR test for *Xcm*, (-) means no observation of symptoms, bacterial streaming or PCR product. Achira, African arrowroot and Indian shot are *Canna* species. <sup>a</sup>The primers used in PCR are from Adriko *et al.* (2012) and Lewis Ivey *et al.* (2010).

Test plant species	Symptoms observation	Ooze test	PCR test <sup>a</sup>		
			BXW	GspDm	NZ085
Achira	+	+	+	+	+
African arrowroot	+	+	+	+	+
Amarants	-	-	-	-	-
Cassava	-	-	-	-	+
Beans	-	-	-	-	+
Enset	+	+	+	+	+
Groundnuts	-	-	+	+	+
Indian shot	+	+	+	+	+
Maize	-	-	+	+	+
Potato	-	-	-	-	-
Pumpkin	-	-	+	+	+
Sorghum	-	-	+	+	+
Sweet pepper	-	-	-	-	-
Taro	-	-	-	-	-
Tomato	-	-	-	-	-
Wheat	-	-	-	-	+
Wild banana	+	+	+	+	+

## 5.2 Potential hosts of the RSSC from potato grown in Rwanda

The RSSC is known to have an unusually wide host range, comprising over 200 host species from more than 50 botanical families (Allen *et al.* 2005),

which gives the pathogen an evolutionary advantage (Hayward, 1991). The host range is continuously expanding and descriptions of new hosts are continuously reported. In this study, we used an isolate belonging to the RSSC phylotype II (Table 6) and which is *R. solanacearum* as per the new classification of the RSSC by Safni *et al.* (2014). A large number of samples collected during 2014 and 2016 from different potato growing areas in Rwanda also revealed the presence of only phylotype II (**paper V**). We evaluated the susceptibility of 14 plant species (Table 5) and potato was used as a positive control. Of all tested plant species, only potato, tree tomato, tomato, eggplant and sweet pepper expressed disease symptoms but at relatively different time points and only potato, tomato and tree tomato wilted completely during the experimental period (Figure 8).

Table 6. Observation of symptoms in the greenhouse, ooze streaming and PCR tests in the plant species inoculated with *R. solanacearum*. (+) denotes observation of symptoms in the greenhouse, bacterial streaming in a glass of water and PCR products for *R. solanacearum*. (-) means no observation of symptoms, bacterial streaming or PCR products.

Test plant species	Symptoms observed	Ooze test	PCR tests			
			Phylotype I	Phylotype II	Phylotype III	Phylotype IV
Amaranthus	-	-	-	+	-	-
Banana	-	-	-	+	-	-
Cassava	-	-	-	+	-	-
Common beans	-	-	-	+	-	-
Cucumber	-	-	-	+	-	-
Eggplant	+	+	-	+	-	-
Groundnut	-	-	-	+	-	-
Maize	-	-	-	+	-	-
Potato	+	+	-	+	-	-
Sorghum	-	-	-	+	-	-
Sweet pepper	+	+	-	+	-	-
Tobacco	-	-	-	+	-	-
Tomato	+	+	-	+	-	-
Tree tomato	+	+	-	+	-	-
Wheat	-	-	-	+	-	-

Potato was the first to show symptoms and to wilt completely of all symptomatic plant species, probably because it is the main host of the bacterial strain used in this experiment. It was followed by tomato and tree tomato which also wilted completely during the experimental period. Eggplant and sweet pepper did not wilt completely. These results agree with the findings by Buddenhagen (1962) who suggested that *R. solanacearum* race 3 strains, originally described as pathogenic on potato and tomato, are weakly virulent on other solanaceous crops. Similarly, Lebeau *et al.* (2011) showed that although eggplant, tomato and pepper belong to the same family, they interact differently with the *R. solanacearum* strains, showing high bacterial wilt resistance in eggplant and pepper but not in tomato. Other test plant species (amaranth, banana, cassava, common beans, cucumber, groundnut, maize,

sorghum, tobacco and wheat) remained healthy along the experimental period and tested negative in the bacterial streaming test (ooze test).

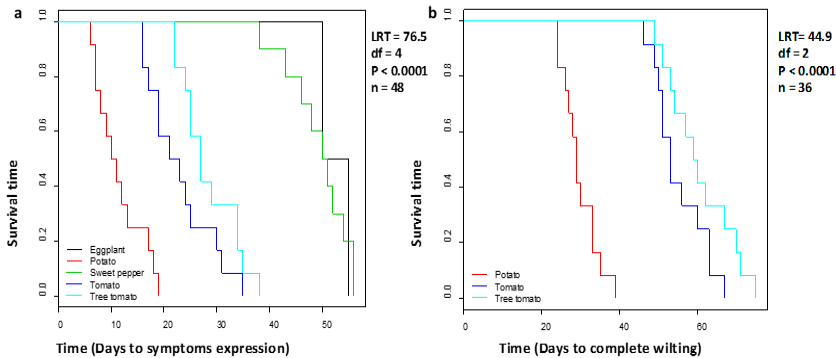


Figure 8. Kaplan Meier estimates for days to symptoms expression (a) and days to complete wilting (b) of symptomatic hosts to *R. solanacearum* in the greenhouse. LRT= Likelihood ration test, df= degree of freedom, n= total number of observations (**Paper IV**).

## 6 Cultivars susceptibility to *X. campestris* pv. *musacearum* and RSSC

All banana cultivars inoculated with *Xcm* developed disease symptoms and wilted completely, but at relatively different time points. There was significant differences in time to symptoms expression (LRT = 48.8, df = 4,  $p < 0.0001$ ) and time to complete wilting (LRT = 254, df = 4,  $p < 0.0001$ ) among tested banana cultivars (**paper III**). Though all the inoculated plants for each cultivar expressed the symptoms of banana xanthomonas wilt earlier than 6 weeks after inoculation the AUDPC varied among cultivars. The lowest value for AUDPC was observed for cultivar Fhia-17 and the highest for cultivar Mpologoma. The cultivar Mpologoma was the first to express disease symptoms and to wilt completely, while the incubation period and time to complete wilting were the longer in cultivar Fhia-17 (**paper III**).

Differences in AUDPC and survival times among banana cultivars could be associated to their differences in genetic groups (Tripathi and Tripathi, 2009). Cultivar Fhia-17, which belongs to the genetic group AAAA, exhibited lower AUDPC and longer survival times than other cultivars, whereas the highly susceptible cultivar Mpologoma belongs to AAA group. East African banana cultivars including Mpologoma, Mbwarzirume/Nkazikamwa and Injagi that belong to AAA-EA genetic group were more susceptible than Fhia-17 in this study. Contrarily, Tripathi and Tripathi (2009) demonstrated less susceptibility to *Xcm* in cultivars Mpologoma and Mbwarzirume compared to Fhia-17. These contradicting findings could be attributed to differences in inoculum dose, age of experimental plants and experimental locations between two studies. Previous studies also demonstrated variations in susceptibility to *Xcm* between banana cultivars (Ssekiwoko et al., 2006b; Tripathi et al., 2008; Tripathi and Tripathi, 2009). Moreover, the high values of AUDPC in all tested cultivars indicate high susceptibility of cultivated banana cultivars (Haynes & Weingartner, 2004).

None of the nine potato cultivars used in this study was found to be resistant to *R. solanacearum* (**paper IV**). However, their reaction to infection varied significantly from cultivar to cultivar. That was demonstrated by differential time to symptoms expression and to complete wilting, AUDPC, as well as the variance in number and weight of tubers produced. Different susceptibility level in potato cultivars to *R. solanacearum* have previously been demonstrated in Kenya (Muthoni *et al.*, 2014; Felix *et al.*, 2011; Ateka *et al.*, 2001b). Our study shows significant differences in days to symptoms expression and days to complete wilting between test cultivars. The first cultivar to show symptom was Kirundo, followed by Gikungu cultivar whereas the cultivars Cruza and Sangema were the last to express disease symptoms. Similarly, the shortest time to complete wilting was observed in cultivar Kirundo, followed by the cultivars Victoria and Mabondo, while the long time to complete wilting were observed in Cruza and Kinigi. Correspondingly, the AUDPC calculated from disease incidence and severity data varied considerably amongst potato cultivars. Based on the combined results on number and weight of tubers produced per potato plant for each cultivar inoculated with *R. solanacearum* (Table 7), the cultivar Cruza remains the least susceptible cultivar followed by cultivars CIP-58 and Kinigi whereas the cultivar Gikungu is the highly susceptible followed by Kirundo and Victoria cultivars (**paper IV**).

Table 7. Means comparison for the number and the weight of harvested tubers per pot by potato cultivars for both inoculated and control treatments. Treatments with the same letter were not significantly different in number of tubers produced and the weight of harvested tubers within a 95% confidence interval; g = grams

Cultivars	Number of tubers/plant		Weight of tubers (g) /plant	
	Inoculated	Control	Inoculated	Control
Cruza	5.3 <sup>a</sup>	7.8 <sup>a</sup>	56.7 <sup>a</sup>	171.8 <sup>b</sup>
CIP-58	4.8 <sup>a</sup>	8.5 <sup>a</sup>	39.3 <sup>b</sup>	196.9 <sup>a</sup>
Kinigi	4.8 <sup>a</sup>	8.5 <sup>a</sup>	39.4 <sup>b</sup>	200.3 <sup>a</sup>
Sangema	4.6 <sup>a</sup>	7.4 <sup>a</sup>	37.7 <sup>b</sup>	181.5 <sup>ab</sup>
Mizero	2.8 <sup>b</sup>	7.3 <sup>a</sup>	18.3 <sup>c</sup>	177.5 <sup>ab</sup>
Mabondo	2.2 <sup>bc</sup>	7.4 <sup>a</sup>	20.0 <sup>c</sup>	176.8 <sup>ab</sup>
Victoria	1.3 <sup>cd</sup>	7.1 <sup>a</sup>	12.6 <sup>c</sup>	178.0 <sup>ab</sup>
Kirundo	1.0 <sup>d</sup>	8.4 <sup>a</sup>	13.8 <sup>c</sup>	189.8 <sup>ab</sup>
Gikungu	0.0 <sup>e</sup>	7.6 <sup>a</sup>	0.0 <sup>d</sup>	187.9 <sup>ab</sup>

Low susceptibility to *R. solanacearum* in potato cultivar Cruza and high susceptibility in cultivar Victoria called Asante in Kenya confirm previous

studies (Muthoni *et al.*, 2014; Ateka *et al.*, 2001b). The tolerance of cultivar Cruza to bacterial wilt has previously been demonstrated in Rwanda (French *et al.*, 1997). This cultivar has also potential tolerance to late blight caused by *Phytophthora infestans* (Muhinyuza *et al.*, 2012). However, Cruza transmit latent infection (infection with no visual symptoms) of potato bacterial wilt (French 1994) and it is not a preferred potato type by the farmers in Rwanda (REMA, 2014).

In conclusion, though some cultivars showed less sensitivity to *R. Solanacearum* and *Xcm* compared to others, there is no good tolerance in extensively multiplied potato and banana cultivars in Rwanda. The breeding program in Rwanda and East and Central Africa would be advised to look for potential sources of resistance in other species like wild *Solanum* species including eggplant for the RSSC (Patil *et al.*, 2012; Lebeau *et al.*, 2011), or wild *Musa* species for *Xcm* (Ocimati *et al.*, 2018).





## 7 Population structure of the pathogen causing potato bacterial wilt

The bacterium *R. solanacearum* species complex (RSSC) is a highly a diverse group of bacterial strains found worldwide and is classified among the most destructive plant pathogenic bacteria (Mansfield *et al.*, 2012). Many of RSSC strains are destructive and cause bacterial wilt disease in a wide range of host plants (Denny, 2007; Hayward, 1994). Based on phylogenetic relationships, the strains in RSSC were subdivided into four phylotypes (I-IV) in relation with their geographical origin and further subdivided into distinct sequevars or sequence variants based on analysis of partial endoglucanase gene sequences (Fegan & Prior, 2005). Each sequevar is further divided into several clonal populations (Subedi, 2015). The phylotyping scheme has recently been employed by a large number of researchers to characterize the populations of RSSC from different parts of the world (Ravelomanantsoa *et al.*, 2018; Albuquerque *et al.*, 2014; Fonseca *et al.*, 2014; Lin *et al.*, 2014; N'Guessan *et al.*, 2013).

Our previous study have revealed that the isolate used to test host range of RSSC belong to the RSSC phylotype II and the host range results indicated that it is the former race 3 biovar 2 (**paper IV**). Recently, another study has shown the presence of biovar 3 from the same area (Mutimawurugo *et al.*, 2019). However these limited samples do not reflect the population structure in the country. Therefore, to assess the population structure of the RSSC in Rwanda, a total of 254 infected potato plant samples were collected from the eight districts surveyed in **paper I**. In addition, 16 infected tomato samples encountered nearby sampled fields were also sampled.

A multiplex PCR reaction (Fegan & Prior, 2005) was used as molecular diagnostic tool to verify the phylotype to which these samples belonged. The results showed that the samples from potato were all RSSC Phylotype II and hence are *R. solanacearum* according to Safni *et al.* (2014) (**paper V**). None of

the potato samples were amplified by any of the other phylotype specific primers. However, phylotype I and III (*R. pseudosolanacearum*) were also recovered from the tomato samples (**paper V**). The multilocus genotyping demonstrated that there is little to no difference in population structure and diversity among potato samples between years, location or cultivar (Figure 9). Two dominating MLGs (multilocus genotypes) were present at all locations during both years.

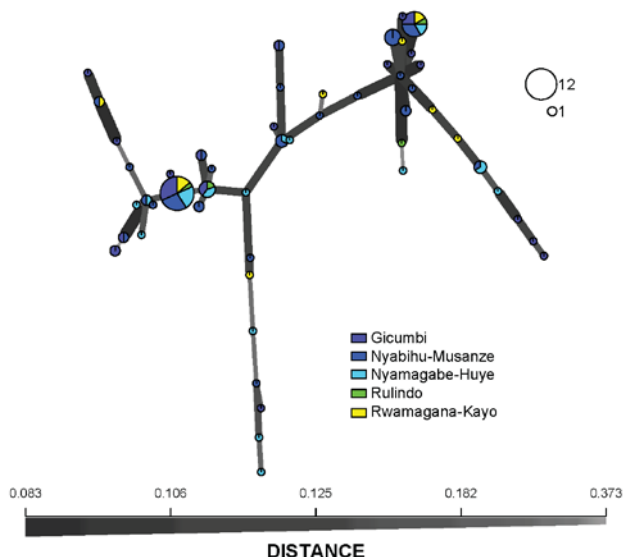


Figure 9. Minimum spanning network based on Bruvo's distance. The colours represent the location in which the samples were collected and the size of the circles reflect the number of samples for the different MLGs.

Phylotype IIB-1 and IIB-2 of *R. solanacearum* are historically known as race 3 biovar 2 (Cellier & Prior, 2010). Race 3 biovar 2 has been previously reported in Rwanda (He *et al.*, 2012). It causes damages in cool climates and is strongly associated with latent infection (French *et al.*, 1998). Ravelomanantsoa *et al.* (2018) reported for the first time phylotype IIB-1 strains in Madagascar and suggested that these strains were associated with potato bacterial wilt outbreaks and that they were introduced and spread through latently infected potato seed tubers. The lack of population structure dominating genotypes reported here (**paper V**) is consistent with spread via potato tubers. Thus improved potato seed production could limit the impact of potato bacterial wilt.

## 8 General conclusions and future perspectives

This is the first detailed study of banana xanthomonas wilt and potato bacterial wilt diseases in Rwanda. Understanding the occurrence, risk factors, host range and cultivars susceptibility is useful to comprehend the diseases epidemiology and for surveillance of the causing pathogens, which would help in the management of these diseases. The main findings of this thesis were:

- ❖ Banana xanthomonas wilt and potato bacterial wilt are widely distributed in Rwanda. This distribution of the diseases was influenced by factors including farm locations, farming practices and farmers' knowledge and attitudes (**paper I and II**).
- ❖ High incidence of banana xanthomonas wilt was positively associated with major banana growing zones, intercropping system, banana grown for brewing, dense and moderate spacing. Similarly, higher banana xanthomonas wilt severity was associated with major banana growing zones, intercropping systems, banana grown for brewing and homogenous cultivation of either improved or indigenous cultivars (**paper II**).
- ❖ High incidence and severity of potato bacterial wilt showed high probability of association with farm tools sharing while low incidence and severity of this disease was highly associated with high altitude, crop rotation and intercropping systems as well as wide spacing between plants (**paper I**).
- ❖ The farmers' knowledge on banana xanthomonas wilt and potato bacterial wilt is scarce and haphazard, which is a result of the quality of information that they use. The major sources of information are fellow farmers, which leads to insufficient disease management attitudes (**paper I and II**).

- ❖ All banana and potato cultivars and their relatives are susceptible to *Xcm* and *R. solanacearum* respectively. Maize, sorghum, pumpkin and groundnuts could be potential sources of inoculum of *Xcm* as proven by PCR. Other crops could also be a source of *R. solanacearum* due to pathogen survival even though the bacterium was not able to cause symptoms in those plants.
- ❖ The RSSC in potato from Rwanda is dominated by phylotype II, *R. solanacearum*, but *R. pseudosolanacearum* (phylotype I and III) was detected in tomato. The population structure of *R. solanacearum* emend. Safni showed little or no variations population structure among samples from different regions.

These findings could provide the baseline of future studies of banana xanthomonas wilt and potato bacterial wilt:

- ❖ In **paper I**, we found that potato planted at higher altitude have lower incidence and severity of potato bacterial wilt, there is a need to test this finding under field conditions.
- ❖ Mechanisms behind the low banana xanthomonas wilt severity in mixed cultivars (**paper II**) should be explored and applicability of this practice needs further investigations.
- ❖ Both *Xcm* and *R. solanacearum* are easily spread through latent infection in seeds and marketed products, hence the movement of potatoes and bananas locally and across borders should be monitored so that the disease outbreaks could be detected on time.
- ❖ Field experiments to screen cultivars of banana and potato under natural field infestation would complement our results.
- ❖ A field test of plant species that harboured the bacterial pathogens (i.e. were positive when tested by PCR) but tested negative in the greenhouse and ooze test, could provide evidence whether these plants could host the pathogens.
- ❖ There is also a need to develop better extension materials for the farmers and improve extension channels.
- ❖ The distribution of the different species in the RSSC need to be further examined along with relevant host range studies.

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## Popular science summary

Rwanda is a small country in the heart of Africa with about 12 million people on 26,336 km<sup>2</sup> surface area including water and nature reserves. Agriculture is the pillar of the country's economy. Banana and potato are important crops for food security and income generation in Rwanda, and they constitute a large share in area cultivated and total production among other crops. Rwanda is among the top five potato producing countries in Africa and the third in sub-Saharan Africa. Banana is grown for cooking, roasting, brewing and direct consumption, as well as income generation in Rwanda. About one quarter of the arable land is allocated to banana because it assures continuous income under conditions of severe land shortage. Globally, potato is the third most consumed food commodity after rice and wheat, and it has been recommended as a food security crop; whereas banana (dessert bananas, cooking bananas and plantains) ranks the sixth on the list of staple crops worldwide.

However, these crops are threatened by important pests and pathogens. These organisms are among the major challenges of crop production worldwide as they cause damage to crops, reduce the availability and access to food and increase the cost for food. Plant diseases caused by the bacteria are highly contagious and difficult to control. This is also true for banana xanthomonas wilt caused by *Xanthomonas campestris* pv. *musacearum* and potato bacterial wilt caused by the *Ralstonia solanacearum* species complex (RSSC).

There is no single control method that can control these diseases, and complete yield loss can occur if proper management strategies are not executed. All the cultivars are vulnerable to the bacteria and regular farming activities by the farmers contribute to the unintentional spread of these bacteria. Both *Xanthomonas campestris* pv. *musacearum* and the RSSC have similar transmission methods and management of their respective diseases rely mostly on cultural practices. To be able to design an appropriate disease

management approach, a good understanding of the disease rate, driving factors, other plant species that can be affected and the susceptibility of the cultivars is required.

The aim of the first paper was to assess the occurrence, risk factors, farmers' knowledge and attitudes of potato bacterial wilt. The results revealed that the disease was present in all the study areas and most challenging in areas where potato is considered a minor crop. The practices such as low plant density, intercropping, crop rotation and avoidance of sharing farm tools occurrence could lower the occurrence of potato bacterial wilt. It was also demonstrated that potato planted at higher altitude have lower incidence and severity of potato bacterial wilt but this need to be tested under field conditions. The knowledge on the detection, spread and management of potato bacterial wilt is scarce and haphazard among interviewed farmers, as a result the management attitudes are unsatisfactory.

The second paper aimed to evaluate the distribution of banana xanthomonas wilt, the associated factors, the farmers' knowledge and actual application of management practices as well as the source of this knowledge. The findings of this study showed that banana xanthomonas wilt has continued to spread compared to previous reports and was present in all the study areas. It was particularly higher in major banana growing areas. The future disease management extension efforts should take into consideration the effects of practices like intercropping, dense spacing, and cultivar homogeneity as well as agro-ecological zone. For the first time, this study reported the link between cultivar mixtures and low disease severity. This practice could be added to the disease management package. Major information providers to the farmers in this study are fellow farmers and close relatives followed by local leaders. The sources of information play an important role on the quality of information shared. If these sources have incorrect or incomplete information, the shared information can be misleading. Hence, it is essential to enhance the information used by information distribution channels in order to ensure that the right information reaches the farmer.

The third paper aimed to verify the ability of the bacterium causing banana xanthomonas wilt to infect crops intercropped with banana, crops grown in neighbouring fields to banana and cultivated banana relatives and to assess the susceptibility level of major banana cultivars. The results showed that the bacterium could only infect banana and banana relatives including wild banana, enset, African arrow root, achira, and Indian shot. Avoidance of cultivating these susceptible plants in close connection to banana plants or fields is recommended to the farmers and flower companies should be aware of the potential threat as well as the possibility to spread the bacterium through



trading infected products. All the cultivars in this study were susceptible to the bacterium, but at slightly different levels. The cultivar FHIA-17 was the least susceptible while cultivar Mpologoma was highly susceptible. Due to the broad range of hosts and high susceptibility of banana cultivars to this bacterium, information about how to limit the spread of the bacterium is crucial for disease control.

Paper four investigated the capability of the bacterium causing potato bacterial wilt to infect plant species grown in the neighbourhood of potato plants and the level of susceptibility to this bacterium among the important potato cultivars. The results revealed that among fifteen test plant species, the bacterium could induce symptoms in potato, tomato, tree tomato, sweet pepper and eggplant only. Only potato, tomato and tree tomato wilted completely. These solanaceous crops should not be grown together or in rotation with potato. All the nine potato cultivars tested were susceptible to the bacterium; however the number of days to symptoms expression, days to complete wilting, area under disease progress curve, and the number and weight of harvested tubers varied considerably among cultivars. Hence, there is a need to look for other sources of resistance.

The fifth paper aimed to evaluate the diversity of the bacteria causing potato bacterial wilt in Rwanda. The results revealed that all samples collected from potato belong to a group called phylotype II. This group contains strains that infect mostly potato and tomato; but are weakly pathogenic on other solanaceous crops. Two groups called phylotype I and III (also known as *R. pseudosolanacearum*) were collected from tomato.

These diseases showed a similar distribution pattern, possibly due the propagation methods of the host plants as well as the difficulty in detecting latent infections. Disease management possibilities and subsequent information channels were also similar. The results from these studies will lead to better disease management methods for producers of these crops, and should lead to more secure food production in Rwanda.



## Populärvetenskaplig sammanfattning

Rwanda är ett litet land i hjärtat av Afrika med omkring 12 miljoner invånare på en yta av 26 336 km<sup>2</sup> inklusive vattendrag och naturreservat. Jordbruk är hörnstenen i landets ekonomi. Banan och potatis är viktiga grödor för livsmedelssäkerhet och möjligheter till inkomst i Rwanda och de upptar en stor andel av den odlade arealen och den totala produktionen av grödor. Rwanda är bland de fem främsta potatisproducerande länderna i Afrika och nummer tre i Afrika söder om Sahara. Banan odlas för kokning, rostning, ölbrygning, och direkt konsumtion och för att skapa inkomster. Omkring en fjärdedel av den odlade arealen upptas av banan eftersom det garanterar en kontinuerlig inkomst under förhållanden då det är brist på odlingsmark. Efter ris och vete är potatis globalt den mest viktigaste livsmedelsråvaran och potatisodling rekommenderas för att öka livsmedelssäkerheten medan bananer (kok-bananer och dessert-bananer) rankas på sjätte plats i världen på listan över grödor som används som basföda.

Både banan och potatis hotas av viktiga skadedjur och patogener. Dessa organismer är några av de största utmaningarna för växtproduktionen i världen eftersom de skadar grödor, minskar tillgängligheten och tillgången till livsmedel och ökar matkostnaderna. Växtsjukdomar orsakade av bakterier kan spridas effektivt och är svåra att bekämpa. Det gäller också *Xanthomonas*-vissnesjuka hos banan som orsakas av *Xanthomonas campestris* pv. *musacearum* och mörk ringröta i potatis orsakad av *Ralstonia solanacearum*.

Det finns ingen enkel metod för att effektivt bekämpa dessa sjukdomar, vilka kan leda till total skördeförlust om bekämpningsåtgärder inte vidtas. Alla banan- och potatissorter är känsliga för angrepp av respektive bakterie och vanliga odlingsåtgärder bidrar, oavsiktligt, till att sprida bakterierna. Både *Xanthomonas campestris* pv. *musacearum* och *Ralstonia solanacearum* har liknande spridningssätt och för att bekämpa de båda sjukdomarna måste man huvudsakligen lita till odlingsåtgärder. För att kunna utforma lämpliga bekämpningsmetoder krävs en god förståelse av spridningsvägar, faktorer som

påverkar spridningen, vilka andra växtarter som kan angripas och känsligheten hos olika sorter.

Avhandlingsarbetet består av fem delstudier. Syftet med den första var att undersöka förekomsten, riskfaktorer, odlarnas kunskaper och attityder kring mörk ringröta i potatis. Resultaten visar att sjukdomen förekom i alla områden som ingick i studien med störst omfattning i områden där potatis odlas i mindre omfattning. Med lägre planttäthet, samodling, växtföljd och genom att undvika att dela odlingsredskap med andra odlare skulle förekomsten av mörk ringröta i potatis kunna minska. I potatis som odlades på högre höjder var förekomsten och angreppsgraden av mörk ringröta lägre, men detta måste undersökas vidare i fält. Kunskapen om detektion, spridning och bekämpning av mörk ringröta i potatis var otillräcklig och slumpmässig bland de intervjuade odlarna, vilket resulterade i att tillämpningen av bekämpningsmetoder var otillfredsställande.

I den andra studien var syftet att undersöka utbredningen av xanthomonas-vissnesjuka hos banan, faktorer förknippade med sjukdomen, odlarnas kunskaper och faktisk tillämpning av odlingsmetoder såväl som varifrån de fick kunskaps om sjukdomen. Den här studien visar att xanthomonas-vissnesjuka hos banan hade fortsatt sprida sig jämfört med tidigare rapporterad utbredning, och förekom i alla studerade områden. Förekomsten var avsevärt högre i områden med där bananodlingen är viktig. Framtida rådgivningsinsatser bör rekommendera bananodlare att undvika samodling, tät plantering och monokulturer utan sortblandning samt att väga in i vilken agro-ekologisk zon odlingen sker. Det här är första gången som ett samband mellan sortblandningar och svagare sjukdomsangrepp har rapporterats. Sortblandning kan komma att ingå som en av flera rekommenderade bekämpningsåtgärder. De huvudsakliga informationskällorna för odlarna i denna studie är andra odlare och nära släktingar följt av lokala ledare. Informationskällorna spelar en viktig roll för kvalitén i informationen som förs vidare. Om källorna har felaktig eller ofullständig information kommer den information som delas att vara felaktig. Det är därför nödvändigt att förbättra informationen via informationsspridningskanaler för att säkerställa att rätt information når odlaren.

Syftet med den tredje studien var att undersöka förmågan hos bakterien som orsakar xanthomonas-vissnesjuka hos banan att infektera grödor som odlas tillsammans med banan eller i angränsande fält och odlade släktingar till banan, samt att undersöka graden av mottaglighet för angrepp hos de vanligaste banansorterna. Resultaten visar att bakterien bara kan infektera banan och släktingar till banan inklusive den vilda bananen, enset och arter i släktet *Canna*. Odlare rekommenderas att undvika odling av dessa mottagliga arter i närheten av bananplantor eller fält och plantskolor och måste bli

medvetna om det potentiella hotet av såväl som risken att sprida bakterien genom handel med infekterade produkter. Alla banansorter i denna studie var mottagliga för bakterien men i något olika grad. Sorten FHIA-17 var minst känslig medan sorten Mpologoma var mycket mottaglig. På grund av det stora antalet av möjliga värdväxter och hög mottaglighet hos banansorter för denna bakterie är information om hur spridningen av bakterien kan begränsas avgörande för att begränsa sjukdomen.

I den fjärde studien undersöktes förmågan hos bakterien som orsakar mörk ringröta i potatis att infektera växtarter som odlades i närheten av potatisplantor och graden av mottaglighet för denna bakterie i viktiga potatissorter. Resultaten visade att bland femton testade arter gav bakterien bara symptom hos potatis, tomat, trädmat, paprika och äggplanta. Endast potatis, tomat och trädmat visade helt. Dessa *Solanum*-arter bör inte odlas tillsammans med eller i samma växtföljd som potatis. Alla nio potatissorter som undersöktes var mottagliga för bakterien men antalet dagar innan symptom uppträdde, antalet dagar till fullständig nedvissning, samt antalet och vikten av skördade potatisknölar varierade avsevärt mellan sorterna. Det är därför viktigt att söka efter andra källor till resistens.

I den femte studien undersöktes diversiteten hos bakterierna som orsakar mörk ringröta i potatis i Rwanda. Resultaten visar att alla prover som samlades in från potatis hör till en grupp som kallas fylogrupp II. Denna grupp innehåller stammar som infekterar främst potatis och tomat, men är svagt patogena hos andra odlade *Solanum*-arter. Två grupper kallade fylogrupp I och III (även kända som *R. pseudosolanacearum*) samlades in från tomat.

De undersökta sjukdomarna uppvisade liknande utbredningsmönster förmodligen beroende på liknande förökningsmetoder hos värdväxterna och svårigheten att upptäcka latent (vilande) infektioner. Bekämpningsmetoder och informationskanaler var också liknande för grödorna. Resultaten från studierna kommer att leda till bättre bekämpningsrekommendationer till odlare av dessa grödor och kan leda till en förbättrad livsmedelssäkerhet i Rwanda.



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May God Bless u all!



# Appendix. Questionnaire for banana growing farmers and farming data recorded

Date: ..... /...../....

Questionnaire No.....

District: ..... Sector..... Cell..... Village: .....

## A. General information

### 1. Household particulars

Farmer' name	Household size	Age	Sex	Marital status	Education	Farming experience	Other Occupation

Codes:

- Respondent marital status: *0–single, 1– married, 2– divorced, 3 – widowed*
- Education: *0-not in school, 1-primary, 2-secondary, 3-vocational training, 4-university*

### 2. Land ownership

Size of land owned (hectares/acres) \_\_\_\_\_

Size of owned land under banana production (hectares/acres) \_\_\_\_\_

Size of land rented (hectares/acres) \_\_\_\_\_

Size of rented land under banana production (hectares/acres) \_\_\_\_\_

Amount of rent paid on rented banana land (rwf /acre/ period) \_\_\_\_\_

### 3. What cultivars do you grow? \_\_\_\_\_

### 4. How do you source planting materials?

- Own farm
- Inter-household (friends)
- Known seed multipliers
- Tissue cultures
- Others

### 5. How do you maintain the new suckers to become planting materials when you want to install a new orchard? \_\_\_\_\_

### 6. What size of suckers do you select for planting? \_\_\_\_\_

### 7. What is the spacing between plants? \_\_\_\_\_

### 8. What major diseases or pests have you been facing in your banana plantation? \_\_\_\_\_

## B. Farmers awareness of banana xanthomonas wilt disease

### 9. If xanthomonas wilt is one of the diseases, in which year did you first hear about it? \_\_\_\_\_

### 10. When did it first appear in your farm? \_\_\_\_\_

➤ **Detection**

11. Do you know how to detect banana bacterial wilt? Yes \_\_\_\_\_ No \_\_\_\_\_  
If yes, how do you detect it?
- 

12. How did you learn how to detect banana xanthomonas wilt? (In the list below)

Sl. No.	Source
1	Radio
2	Television
3	MINAGRI
4	RAB Scientists
5	NGOs
6	Researchers
7	Extension officer
8	Local leader
9	Agronomists
10	Training (demonstration, seminar, workshop)
11	Posters
12	Brochures
13	News papers
14	Fellow farmers
15	Parents

13. When did you first learn of the detection methods for xanthomonas wilt in banana? \_\_\_\_\_

➤ **Spreading**

14. Do you know how xanthomonas wilt spreads? Yes \_\_\_\_\_ No \_\_\_\_\_  
15. If yes, how does it spread
- 

16. How did you learn how it spreads (among the sources below)?

Sl. No.	Source
1	Radio
2	Television
3	MINAGRI
4	RAB Scientists
5	NGOs
6	Researchers
7	Extension officer
8	Local leader
9	Agronomists
10	Training (demonstration, seminar, workshop)
11	Posters
12	Brochures
13	News papers
14	Fellow farmers
15	Parents

17. When did you first learn how xanthomonas wilt spreads? \_\_\_\_\_

➤ **Control**

18. Do you know how to control xanthomonas wilt? Yes \_\_\_\_\_ No \_\_\_\_\_
19. If yes, how do you control it? \_\_\_\_\_
20. How did you learn how xanthomonas wilt is controlled (among the sources below)?

Sl. No.	Source
1	Radio
2	Television
3	MINAGRI
4	RAB Scientists
5	NGOs
6	Researchers
7	Extension officer
8	Local leader
9	Agronomists
10	Training (demonstration, seminar, workshop)
11	Posters
12	Brochures
13	News papers
14	Fellow farmers
15	Parents

21. When did you first learn how banana bacterial wilt is controlled? \_\_\_\_\_

**C. Implementation of banana xanthomonas wilt management practices**

22. Do you remove male buds to your banana plants? Yes \_\_\_\_\_ No \_\_\_\_\_
23. Do you sterilize your farm tools? Yes \_\_\_\_\_ No \_\_\_\_\_
- If yes, how and when do you sterilize your farm tools?
24. Do you share your farm tools with your friends? Yes \_\_\_\_\_ No \_\_\_\_\_
25. Do you use your farm tools in someone else's farm? Yes \_\_\_\_\_ No \_\_\_\_\_
26. Do you exchange planting materials with your friends or neighbours?  
Yes \_\_\_ No \_\_\_\_\_
27. Have you ever experienced the disease incidences in banana suckers?  
Yes \_\_\_\_\_ No \_\_\_\_\_
28. What do you think caused the problem? \_\_\_\_\_
29. How do you prevent the reoccurrence of the problem? \_\_\_\_\_

**D. Data collected from the field with regard to banana xanthomonas wilt disease**

1. Farm location (GPS Coordinates)
2. Cropping system
  - a. Monocropping (Pure stand)
  - b. Intercropping
3. Crops intercropped with banana or crops in the neighbouring fields \_\_\_\_\_

4. Types of grown cultivars
  - a. Brewing
  - b. Cooking
  - c. Dessert
5. Cultivars characteristics
  - a. Indigenous
  - b. Improved
  - c. Mixed
6. What is the spacing between banana mats
  - a.  $< 1\text{m}^2$
  - b.  $1\text{m}^2 - 2\text{m}^2$
  - c.  $> 2\text{m}^2$